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Repurposing FDA-approved drugs against multiple proteins of SARS-CoV-2: An *in silico* study

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ABSTRACT

The current crisis of the COVID-19 pandemic around the world has been devastating as many lives have been lost to the novel SARS CoV-2 virus. Thus, there is an urgent need for the right therapeutic drug to curb the disease. However, there is time constraint in drug development, hence the need for drug repurposing approach, a relatively fast and less expensive alternative. In this study, 1,100 Food and Drug Administration (FDA) approved drugs were obtained from DrugBank and trimmed to 791 ligands based on illicitness, withdrawal from the market, being chemical agents rather than drugs, being investigational drugs and having molecular weight greater than 500 (Kg/mol). The ligands were docked against six drug targets of the novel SARS CoV-2 - 3-chymotrypsin-like protease (3CLpro), Angiotensin-converting enzyme (ACE2), ADP ribose phosphatase of NSP3 (NSP3), NSP9 RNA binding protein (NSP9), RNA dependent RNA polymerase (RdRp) and Replicase Polyprotein 1a (RP1a). UCSF Chimera, PyRx and Discovery Studio, were used to prepare the proteins, dock the ligands and visualize the complexes, respectively. Remdesivir, Lopinavir and Hydroxychloroquine were used as reference drugs. Pharmacokinetic properties of the ligands were obtained using AdmetSAR. The binding energies of the standard drugs ranged from -5.4 to -8.7 kcal/mol while over 400 of the ligands screened showed binding energy lower than -5.4 kcal/mol. Out of the 791 number of compounds docked, 10, 91, 132, 92, 54 and 96 compounds showed lower binding energies than all the controls against 3CLPro, ACE2, NSP3, NSP9, RP1a and RdRp, respectively. Ligands that bound all target proteins, and showed the lowest binding energies with good ADMET properties and particularly showed the lowest binding against ACE2 are ethynodiol diacetate (-15.6 kcal/mol), methylnaltrexone (-15.5 kcal/mol), ketazolam (-14.5 kcal/mol) and naloxone (-13.6 kcal/mol). Further in-

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vestigations are recommended for ethynodiol diacetate, methylnaltrexone, ketazolam and naloxone through preclinical and clinical studies to ascertain their effectiveness.

Introduction

The coronavirus disease (COVID-19) pandemic caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) is currently a global public health concern [1]. It is transmitted from one person to another via small droplets from coughing, sneezing, talking or contact with contaminated surfaces. The present situation report shows that there have been over 23 million cases, and over 840,000 deaths from COVID-19 globally; no effective treatment or vaccine has been found [2, 3]. Coronavirus belongs to the Coronaviridae family. There are four subfamilies of the virus, classified as Beta-coronavirus, Gamma-coronavirus, Alpha-coronavirus and Delta-coronavirus. SARS CoV-2 belongs to Beta-coronavirus [4]. It has been reported that Alpha- and Beta-coronaviruses are predominant in bats and rodents whereas Delta- and Gamma-coronavirus gene sources are the avian species [5].

The life cycle of coronavirus has shown potential drug targets essential for its survival. Some of the viable drug targets include a 3-chymotrypsin-like protease (3CLpro), Angiotensin-converting enzyme (ACE2), ADP ribose phosphatase of NSP3 (NSP3), NSP9 RNA binding protein (NSP9), RNA dependent RNA polymerase (RdRp) and Replicase polyprotein 1a (RP1a). For host infectivity, the structural spike glycoprotein interacts with the transmembrane protein of the human host cell receptor ACE2 [6]. The virus then progresses into the endosomes where structural changes occur in the spike glycoprotein that allows the virus into the human host cell. Afterwards, RdRp enables the viral genome replication using cofactors such as nsp7 and nsp8 [7]. The main protease 3CLpro elicits the proteolytic cleavage of the viral protein RP1a into functional units, which then produces nonstructural proteins - nsps (including NSP3 and NSP9). These nsps are involved in the replication and transcription of the virus.

The main protease 3CLpro acts in the proteolytic cleavage of the viral polyprotein large replicase polyprotein 1a (RP1a), cleaving it into functional units to produce nonstructural proteins (nsps including NSP3 and NSP9) responsible for the replication and transcription of the virus [8]. Thus, the main protease 3CLpro plays a crucial role in the viral infection cycle and establishment as it aids the production of nonstructural viral constituents as RdRp, NSP3 and NSP9. This makes 3CLpro and ACE2 critical drug targets to stop the production of nsps and establishment of the virus respectively. RP1a, the nsps – RdRp, NSP3 and NSP9 are important targets involved in the replication and translation which lead to viral proliferation in host cells [9, 10]. Since no drugs are available yet to treat this deadly disease, scientists have suggested the use of broad-spectrum antiviral drugs as a promising treatment regimen. Some antiviral drugs like favinapir, ritonavir, oseltamivir, lopinavir, ganci-clovir and remdesivir have been clinically tested against COVID-19 infection. Remdesivir, an antiviral agent, is the only drug approved for the treatment of COVID-19 in certain situations by FDA. [11, 12]. Since no precise treatment is available for COVID-19, drug repurposing of drugs with known pharmacokinetics is a useful approach.

The concept of drug repurposing or drug repositioning seeks new therapeutic uses of pre-existing medications with minimal or no side effects. Research has shown that drug repurposing is largely advantageous because it could significantly shorten the time and reduce the cost of drug discovery and development compared to *de novo* drug discovery and randomized clinical trials [13, 14]. Success stories of drug repurposing have been told of aspirin, sildenafil, minoxidil, valproic acid etc. Therefore, drug repurposing could be a quick alternative to find a treatment for the COVID-19 pandemic. One approach employed in carrying out drug repurposing includes high throughput screening of compound libraries for potent drugs that show potentials.

High throughput screening technology can be used to screen large libraries of potential and existing drugs to support drug repurposing efforts [15]. Virtual screening has been a very useful technique for researchers. It is a quick and economical technique in the drug discovery pipeline [16]. It is used to identify novel hit molecules from large chemical libraries using computational approach. Virtual screening applies the knowledge of the protein target and ligands. Ligand-based screening and structure-based screening are two approaches used in virtual Screening. The most commonly used virtual screening technique is molecular docking [17]. Molecular docking predicts the binding of a ligand to a protein to form stable complexes [18]. Thus, docking is a molecular modeling technique that is used to predict how a protein interacts with small molecules. Also, Lack of efficacy and safety are the two major causes leading to drug failure, which means the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of chemicals play vital roles in every stage of drug discovery and development. Therefore, it is necessary to find efficacious molecules with better ADMET properties [37].

The urgency for the treatment of COVID-19 has necessitated the search for an effective drug that can elicit a positive physiological response on the diseased body. Identifying therapeutically potent drugs from the library of already existing drugs has become expedient since the process for discovering and developing a new drug is not only costly but time-consuming. Repurposing of drugs from their original applications to a totally new application has been reported widely in

literature and in this study, it has been considered a relatively fast and less expensive approach to find therapeutic agents for COVID-19 [19]. This study was carried out to identify potent inhibitors against SARS CoV-2 potential drug targets from libraries of FDA-approved drugs using drug repurposing approach.

Computational methods

Identification and preparation of compounds for docking

The 2D structures of 1,100 Food and Drug Administration (FDA) approved drug candidates were retrieved from DrugBank [20]. Certain other criteria such as illicitness, withdrawal from the market, being a chemical agent rather than drugs, being investigational and having molecular weight greater than 500 were further used to screen the FDA-approved drugs to a sizable number of 791 before docking. Hydroxychloroquine (CID 3652), lopinavir (CID 92727), and remdesivir (CID 121304016) were retrieved from PubChem and used as reference drugs [21]. The DrugBank drugs were converted to Structured Data Format (SDF) using Data warrior. Using the OpenBabel intergration in PyRx, the SDF file was loaded and the structure of the ligand was displayed. The PyRx software (https://pyrx.sourceforge.io/) was used to prepare the ligands for docking. Conjugate gradient algorithm was used to perform energy minimization with the universal force field (uff). The number of steps was set at 200 with the number of updates set at 1. The minimization was set to stop at an energy difference of less than 0.1 kcal/mol. The ligands were further converted to AutoDock ligands format (PDBQT) before multiple docking was carried out.

Identification and preparation of SARS CoV-2 molecular targets

Six SARS CoV-2 molecular targets, 3-chymotrypsin-like protease (PDB ID: 6LU7), Angiotensin-converting enzyme (PDB ID: 6LZG), ADP ribose phosphatase of NSP3 (PDB ID: 6VXS), NSP9 RNA binding protein (PDB ID: 6W4B), RNA dependent RNA polymerase (PDB ID: 7BTF) and Replicase polyprotein 1a (PDB ID: 6YHU) were identified from literature and downloaded from Protein Data Bank [22]. The targets were prepared by removing hetatoms (water and ligands) from the receptors using Biovia Discovery Studio 4.5 software (https://discover.3ds.com/discovery-studio-visualizer-download). The targets were further processed and minimized using UCSF Chimera (https://www.cgl.ucsf.edu/chimera/download.html) by further deleting all nonstandard residues like water and ligands on protein targets. Hydrogen was added, and charges were equally assigned to proteins using Gasteiger charge. The proteins in pdb format were later converted to PDBQT format to be used in the integrated Autodock tool for docking.

Determination of active sites

The active sites of the proteins were determined using The Computed Atlas for Surface Topography of Proteins (CASTp). CASTp is a web-based tool that is used in predicting active sites of a specific protein, as it shows the amino acid residues present in the active site of the target protein [23]. The protein data bank file uploaded into CASTp site and the top results from the potential ligand-binding sites was selected to be used for docking. This was used to identify the active sites of proteins as well as determining the amino acid residues present in our proteins and cross referenced with other literatures [38-41].

Molecular docking using PyRx

The multiple docking of the ligands and proteins were done with Autodock Vina integrated in the PyRx software. PyRx is a Virtual Screening software for computer-based drug discovery that can be used to screen libraries of compounds against potential drug targets. Not all docking softwares can be used for multiple docking as PyRx (https://pyrx.sourceforge.io/). Likewise the integrated autodock vina is much faster and efficient in optimization and multithreading. It calculates the grid charges internally and set up the docking space [42]. After the preparation of both the ligands and the targets, the docking was run with Vina Wizard. The residues on active site of the target protein were selected to set grid box and vina was run to complete the process of docking. The results were downloaded in .csv format which contained both the binding energy and RMSD of the docked complex. Visualization of docked complex was done using discovery studio application. The visualization results showed the 2D structures and bond interactions of the docked protein-ligand complex. Hydroxychloroquine, lopinavir and remdesivir were used as standards for the docking.

Absorption, distribution, metabolism elimination and toxicity (ADMET) studies

The ADMET properties play key roles in the discovery/development of drugs, pesticides, food additives, consumer products and industrial chemicals [43]. The best 8 ligands that showed the least binding energies were selected, canonical smiles prepared and submitted to the swissADME [48] and admetSAR [24] servers to examine their druglike properties as well as different pharmacokinetics and pharmacodynamic parameters. AdmetSAR is a comprehensive free web service tool for predicting Absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of chemicals [49]. SwissADME is

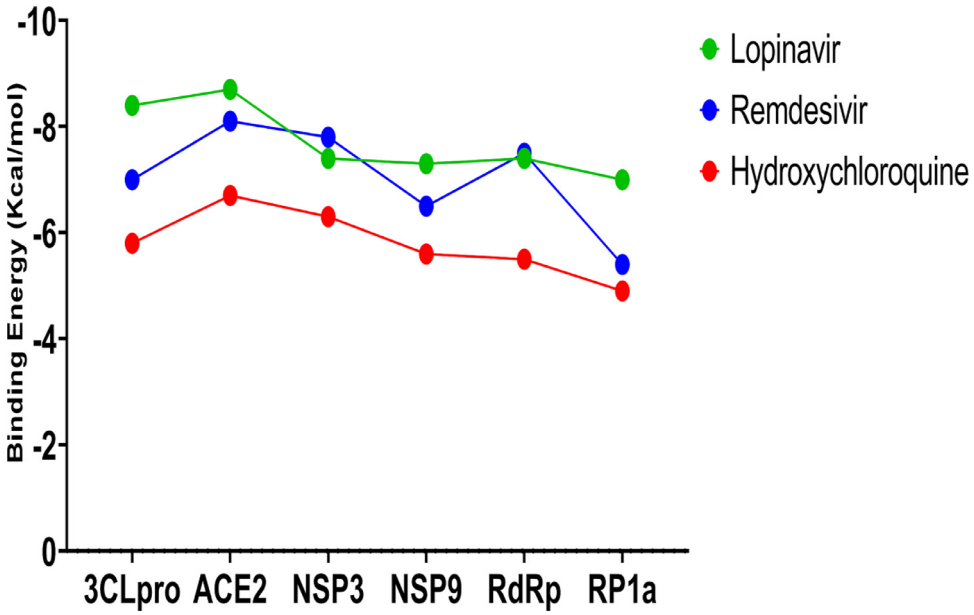

Fig. 1. Binding energies (ΔG) in Kcal/mol of standards drugs with the target proteins. Showing that the standard drugs bound with energies ranging from -8.7 to -5.4 Kcal/mol. The lowest binding energies were seen in Lopinavir-ACE-2 complex (-8.7 Kcal/mol), Remdesivir-ACE-2 complex (-8.1 Kcal/mol), and Hydroxychloroqine-ACE-2 complex (-6.7 Kcal/mol). while the highest binding energies were seen in Hydroxychloroquine-RP1a complex (-4.9 Kcal/mol), Remdesivir-RP1a complex (-5.4 Kcal/mol) and Lopinavir-RP1a complex (-7.0 Kcal/mol).

also a web tool that gives free access to a pool of fast yet vigorous predictive models for physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness, among which in-house proficient methods such as the BOILED-Egg, iLOGP and Bioavailability Radar [50].

Analysis of ligand-protein interactions

Hydrogen bonding and hydrophobic interactions between the ligands and amino acids residues of the protein-ligand complex interactions for the best five ligands that displayed lowest binding energy were visualized using Biovia Discovery studio 4.5 and UCSF Chimera softwares.

Analysis of molecular docking results

The molecular docking results were viewed and organized on spreadsheet. This was further analysed using the Seaborn package in Python and GraphPad Prism 8 for windows version 8.4.3 [25].

Results

Protein targets active sites

The Table SMI shows the active sites of the protein targets as predicted by the Computed Atlas of Surface Topography of protein (CASTp).

Molecular docking of drug compounds against target proteins

The binding energy of the standard drugs displayed in Fig. 1 shows that hydroxychloroquine, lopinavir and remdesivir had lowest binding energy with RP1a. The relative binding energies of these standards were used as the point of reference for the FDA Drug library screened. A cluster of drugs docked with the target proteins is shown in Fig. SM2. Binding energies of some of the ligands topping the list of ligands with low binding energies are reported in Table 1. From the pool of the 791 drugs docked, about 8.4% of the drugs, which represented the ligands with least binding energies, had binding energy ranging from -15.6 to -7.4 kcal/mol. Ethynodiol diacetate-ACE2 had the lowest binding energy while buclizine/ calcitriol/ canagliflozin/ dasabuvir/ metergoline/ netarsudil - RP1a complexes had the highest binding energies. Whereas the standard drugs had binding energies ranging from -8.7 to -5.4 kcal/mol; lopinavir-ACE-2 complex exhibiting the lowest binding energy and hydroxychloroquine-NSP9 complex having the highest binding energy (Fig. 1). The top 10 compounds with the lowest

Table 1
Top 10 drugs with lowest binding energies for target proteins.

S/N	TARGETS	LIGANDS	BINDING AFFINITY (Kcal/mol)
		Ketazolam	-10.6
1	3CLpro	Methylnaltrexone	-10.4
		Ethynodiol diacetate	-10.2
		Naloxone	-9.6
		Lumacaftor	-9
		Idarubicin	-8.8
		Perampanel	-8.8
		Vorapaxar	-8.8
		Paliperidone	-8.6
		Spironolactone	-8.5
		Ethynodiol diacetate	-15.6
2.	ACE2	Methylnaltrexone	-15.5
		Ketazolam	-14.5
		Naloxone	-13.6
		Dicoumarol	-10.7
		Buclizine	-10.3
		Pimozide	-10.3
		Butenafine	-10.2
		Estrone	-10.1
		Etravirine	-10.1
3.	NSP3	Methylnaltrexone	-11.8
		Ethynodiol diacetate	-11.4
		Ketazolam	-11.4
		Naloxone	-10.4
		Eltrombopag	-9.7
		Cromoglicic acid	-9.6
		Regorafenib	-9.6
		Lumacaftor	-9.5
		Bicalutamide	-9.4
		Folic acid	-9.4
4.	NSP9	Methylnaltrexone	-11.3
		Ethynodiol diacetate	-10.5
		Ketazolam	-10.4
		Naloxone	-9.5
		Drospirenone	-8.6
		Spironolactone	-8.6
		Lumacaftor	-8.4
		Netarsudil	-8.4
		Tolvaptan	-8.4
		Vorapaxar	-8.4
5.	RdRp	Ketazolam	-11.7
		Methylnaltrexone	-11.3
		Ethynodiol diacetate	-11.2
		Naloxone	-10.5
		Lumacaftor	-9.4
		Olaparib	-9.2
		Quinestrol	-9
		Rupatadine	-8.9
		Fluprednisolone	-8.8
		Benzhydrocodone	-8.7
	RP1a	Methylnaltrexone	-10.7
6		Ethynodiol diacetate	-10.5
		Ketazolam	-9.5
		Naloxone	-9.3
		Adapalene	-8.3
		Lumacaftor	-8
		Vorapaxar	-8
		Drospirenone	-7.7
		Methyltestosterone	-7.6
		Rupatadine	-7.6

binding energy for each protein target was collated to 31 compounds as there are compounds which occurred for multiple protein targets (Table 1), four ligands - ethynodiol diacetate, methylnaltrexone, ketazolam and naloxone, were found to have the lowest binding energies and had interactions with all the 6 protein targets (Fig. 2). In addition, lumacaftor was found to bind 4 target proteins while vorapaxar bound 3 target proteins. Spironolactone, drospirenone and rupatadine were found to bind 2 out of the 6 targets and 19 ligands bound against one target each with low binding energies. In general, 1.3% of the total ligands were observed to have lower binding energies than controls when the 791 compounds were docked against

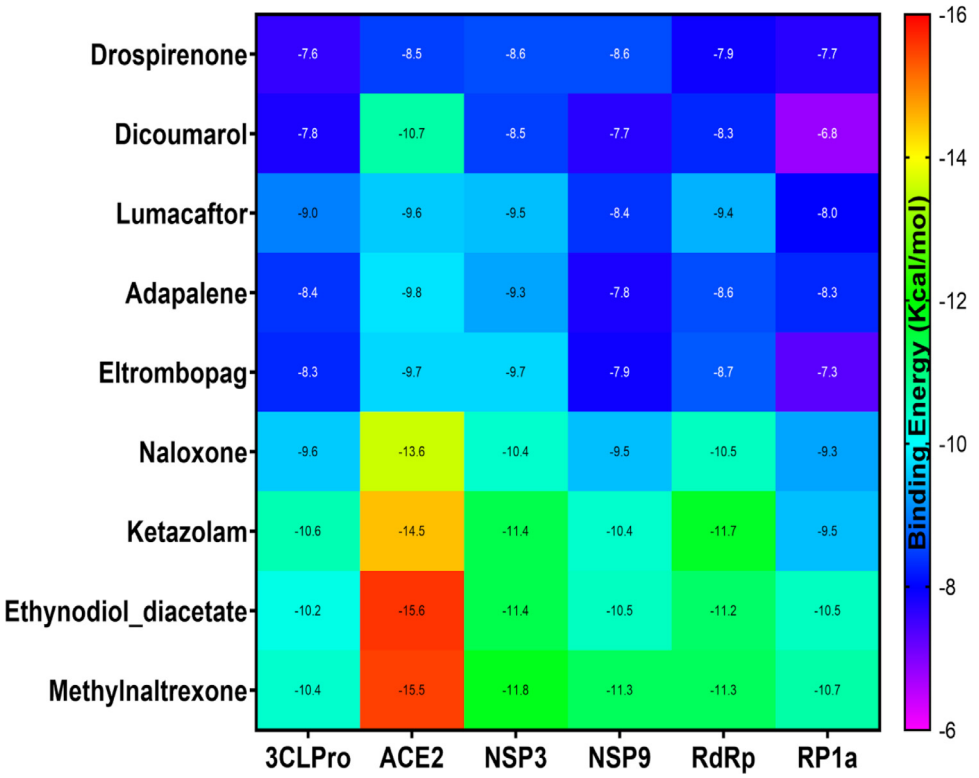

Fig. 2. Molecular Docking Result of top 5 drugs with lowest binding affinities across each protein. Showing that Methylnaltrexone, Ethynodiol diacetate, Ketazolam and Naloxone drugs had lower binding energies than other drugs against 3CLPro, ACE2, NSP3, NSP9, RdRp and RP1a.

3CLpro. Similarly, 11.5%, 16.7%, 11.6%, 6.8% and 12.1 % compounds out of the total ligands were observed to have lower binding energies than controls when docked against ACE2, NSP3, NSP9, RP1a and RdRp, respectively. These compounds were found to have lower binding energies than the standards as shown in Fig. 1 and Table SM1. The seaborne heatmap further showed other hit compounds in the study (Fig. 3).

Absorption, distribution, metabolism, excretion and toxicity (ADMET) analyses of the selected ligands

The prediction of the Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties that revealed the pharmacokinetics of the selected compounds shows the Drug Likeness of the drugs. According to Lipinski's rule of five, it is determined by a complex balance of various molecular properties and structural features such as lipophilicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility, and presence of various pharmacophoric features which in turn influence the behavior of a molecule in a living organism. A good drug candidate should not violate more than one of the rules [47]. The human intestinal absorption, blood brain barrier penetration, P-glycoprotein substrate and inhibitor, and renal organic cation transproter were evaluated and recorded in Table 2. According to the AdmetSAR server prediction, compounds with BBB% and HIA% less than 30% is labelled as (-), therefore ethylnodiol diacetate, dicoumarol and drospirenone; methylnaltrexone were predicted to have low BBB penetration and HIA respectively. The two effector sites (Mitochondrion and Lysosome) were predicted for the drugs from this study. Mitochondrion was predicted to be the effector site for about 88% of the drugs while lysosome is predicted to be the effector site for only methylnaltrexone (Table 2). According to the Ames test prediction result, none of the drugs exhibit acute toxicity and mutagenic effect while the carcinogenicity profile revealed adapalene to be in the "warning class".

All the drugs are non-substrates and non-inhibitors of CYP2C9 and CYP2D6 respectively. Methylnaltrexone and naloxone are substrate of CYP2D6 while lumacaftor, adapalene and dicoumarol are non-substrate of CYP3A4. Lumacaftor is an inhibitor of CYP1A2, CYP2C9 and CYP2C19. Ketazolam is the only inhibitor of CYP3A4 among the test subjects. Only lumacaftor exhibit high CYP inhibitory promiscuity. All the drugs satisfied Lipinski's rule.Our results show that all of the selected ligands (methylnaltrexone, ethynodiol diacetate, ketazolam, naloxone, lumacaftor, eltrombopag, adapalene, dicoumarol, drospirenone) displayed good ADMET properties.

Table 2
Absorption, distribution, metabolism, excretion and toxicity (admet) analysis of the selected ligands.

Ligands	Methylnaltrexone	Ethylnodiol diacetate	Ketazolam	Naloxone	Lumacaftor	Eltrombopag	Adapalene	Dicoumarol	Drospirenone
MW	356.4	384.5	368.8	327.4	452.4	442.5	412.5	336.3	366.5
AlogP	1.67	4.43	3.28	1.30	4.75	3.78	6.68	2.90	4.31
H-Bond Acceptor	4	4	3	5	5	6	2	6	3
H-Bond Donor	2	0	0	2	2	3	1	2	0
Rotatable Bonds	2	2	1	2	5	5	4	2	0
CaCo-2	+	-	+	+	-	-	-	-	+
	0.6133	0.5577	0.8782	0.5760	0.8593	0.7074	0.6084	0.7577	0.6058
Acute Oral	III	III	III	III	III	III	III	II	III
Toxicity	0.5463	0.7844	0.7703	0.6217	0.5320	0.6890	0.5939	0.7149	0.7290
Tetrahymena pyriformis [Pigc50(ug/L)]	1.508	0.912	2.091	2.121	1.226	0.194	1.2	1.31	1.27
BBB	+	-	+	+	+	+	+	-	-
	1.0000	0.3286	1.0000	1.0000	0.9714	0.9627	0.7754	0.9064	0.7071
HIA	-	+	+	+	+	+	+	+	+
	0.8138	0.9907	0.9965	0.9729	0.9806	0.9537	1.0000	0.8724	1.0000
P-gp Substrate	Substrate	Substrate	Substrate	Substrate	Non	Non	Non	Non	Substrate
	0.9279	0.5754	0.7006	0.8788	0.5512	0.7717	0.5408	0.5073	0.6524
P-gp Inhibitor	Non	Inhibitor	Non	Non	Non	Non	Inhibitor	Non	Non
	0.9775	0.8432	0.8837	0.8382	0.6927	0.7879	0.5000	0.8972	0.6726
Renal Organic Cation Transporter	Non	Non	Non	Inhibitor	Non	Non	Non	Non	Non
	0.5377	0.8131	0.7534	0.5000	0.9532	0.9438	0.8646	0.8982	0.7005
Subcellular Localization	Lysosome (0.4753)	Mitochondria (0.7934)	Mitochondria (0.4979)	Mitochondria (0.5800)	Mitochondria (0.7053)	Mitochondria (0.8119)	Mitochondria (0.9264)	Mitochondria (0.8402)	Mitochondria (0.6873)
Aqueous Solubility	-2.5784	-5.1201	-3.9912	-2.5630	-4.2931	-3.4701	-4.0179	-3.1724	-3.7781
CYP2C9	Non	Non	Non	Non	Non	Non	Non	Non	Non
substrate	0.7889	0.8497	0.8193	0.8522	0.7860	0.6108	0.7171	0.8264	0.7960
CYP2D6	Substrate	Non	Non	Substrate	Non	Non	Non	Non	Non
substrate	0.6417	0.9139	0.8504	0.5296	0.8348	0.8564	0.8925	0.9116	0.9116
CYP3A4	Substrate	Substrate	Substrate	Substrate	Non	Substrate	Non	Non	Substrate
substrate	0.6999	0.7647	0.7504	0.6107	0.5056	0.5358	0.5116	0.7557	0.6964
CYP1A2	Non	Non	Non	Non	Inhibitor	Non	Non	Non	Non
inhibitor	0.8884	0.9045	0.5878	0.9046	0.5194	0.9035	0.7812	0.7905	0.5534
CYP2C9	Non	Non	Inhibitor	Non	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Non
inhibitor	0.9271	0.6524	0.5154	0.9303	0.5691	0.5327	0.7758	0.8948	0.8665
CYP2D6	Non	Non	Non	Non	Non	Non	Non	Non	Non
inhibitor	0.6213	0.9519	0.9257	0.7886	0.8092	0.8685	0.9466	0.9681	0.9336
CYP2C19	Non	Non	Inhibitor	Non	Inhibitor	Non	Non	Non	Non
inhibitor	0.8552	0.7671	0.5938	0.9025	0.5471	0.8572	0.6530	0.6071	0.7754
CYP3A4	Non	Non	Inhibitor	Non	Non	Non	Non	Non	Non
inhibitor	0.9521	0.6564	0.6037	0.9153	0.6888	0.8839	0.8246	0.9098	0.8355
CYP inhibitory promiscuity	Low	Low	Low	Low	High	Low	Low	Low	Low
	0.9803	0.8322	0.5200	0.9523	0.5464	0.5491	0.8509	0.9165	0.8541
Ames toxicity	Non	Non	Non	Non	Non	Non	Non	Non	Non
	0.6301	0.9651	0.7228	0.7182	0.6875	0.5666	0.8575	0.9048	0.9163
Carcinogenicity (three class)	Non required	Non required	Non required	Non required	Non required	Non required	Warning	Non required	Non required
	0.5396	0.6566	0.6007	0.5181	0.4184	0.4756	0.4762	0.6978	0.5409

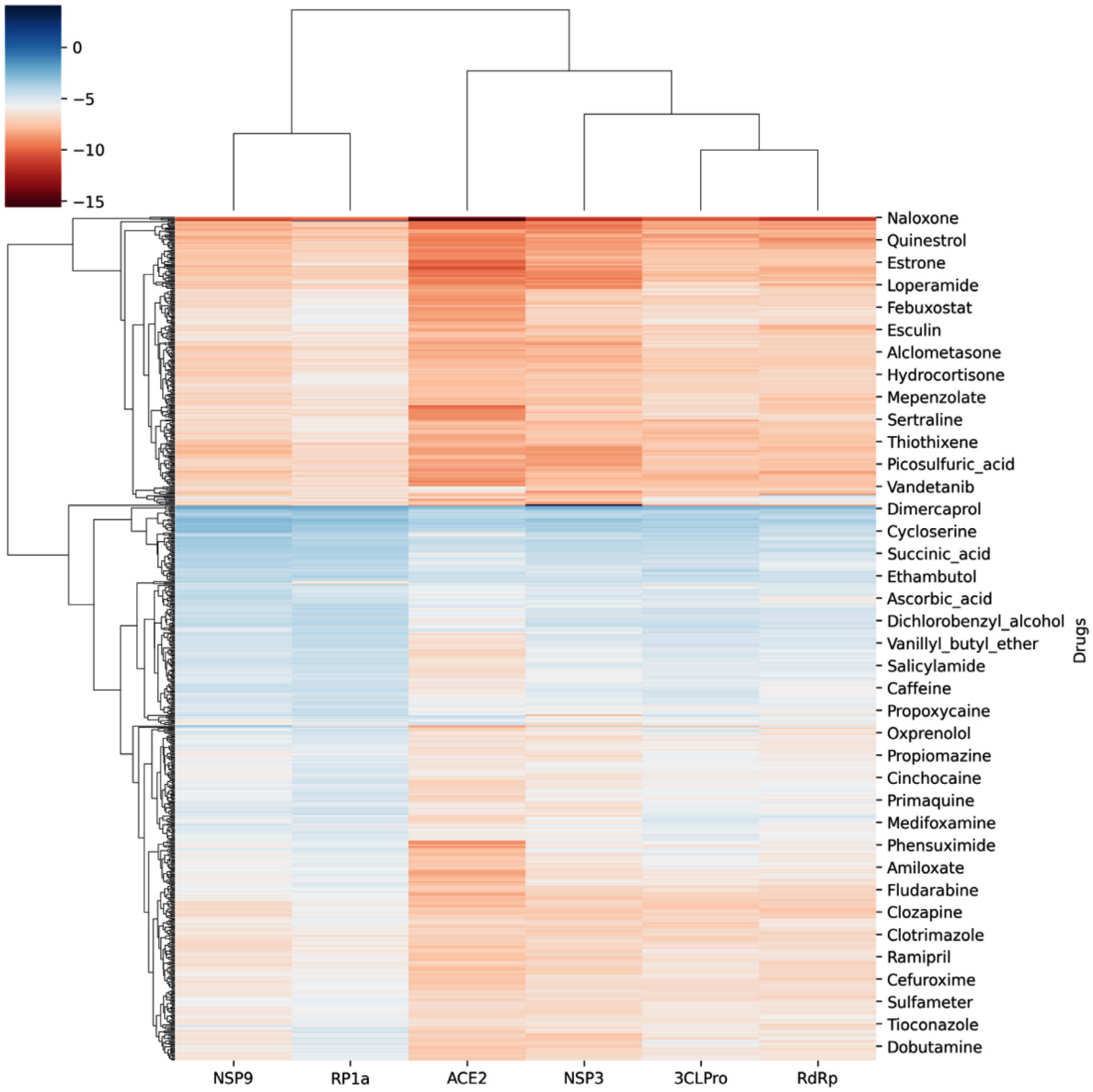

Fig. 3. Cluster map of drugs docked against the target proteins.

Protein-Ligand interactions of the selected drugs and SARS CoV-2 drug targets

The Ligand-protein interactions with the lowest binding energy of each target, which did not violate Lipinski's rule of 5 are further analysed below.

Protein-Ligand interactions between 3-chymotrypsin-like protease and Ketazolam

The ligand ketazolam with binding energy -10.6 kcal/mol formed one conventional hydrogen bond between its oxazino derivative and SER 158 in the 3-CLpro binding site. Nine of the amino acid residues, PHE 8, ASP 153, THR 111, PHE 294, GLN 110, ILE 106, VAL 104, ASN 151, LYS 102 were involved in van der Waals interactions with the phenyl groups of the ligand. (Fig. 4a).

Protein ligand interactions between Ethynodiol diacetate and ACE2–angiotensin-converting enzyme

Ethynodiol diacetate is the ligand with least binding energy (-15.6 kcal/mol) among the group of ligands docked with ACE2. Its interaction with the target showed the presence of one conventional hydrogen bond between its carboxylic group and ILE 291 residue in the ACE2 binding site. The ligand also formed seven van der Waals interactions between

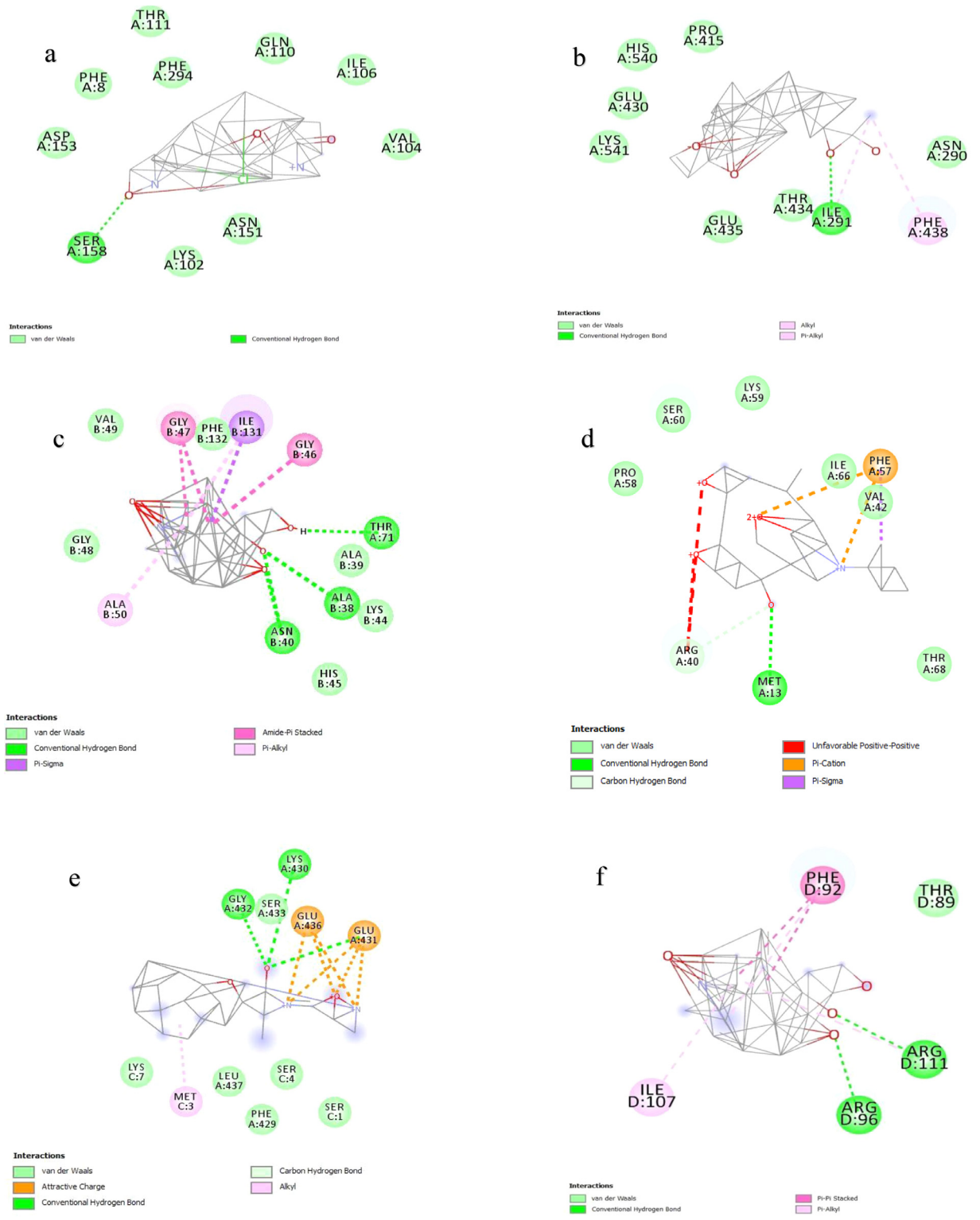

Fig. 4. 2D Protein-Ligand interaction of 3CLpro and Ketazolam (a); ACE2 and Ethynodiol diacetate (b); ADP ribose phosphatase of NSP3 and Methylnaltrexone (c); NSP9 RNA binding protein and Methylnaltrexone (d); RNA dependent RNA polymerase and Ketazolam (e); Replicase polyprotein 1a and Methylnaltrexone (f).

the phenantrenyl group and the amino acid residues PRO 415, HIS 540, GLU 430, LYS 541, GLU 485, THR 434 and ASN 290. Other bonds formed between its methyl group and amino residue PHE 438 include pi- alkyl interaction (Fig. 4b).

Protein-ligand interactions between ADP ribose phosphatase of NSP3 and Methylnaltrexone

The interaction between methylnaltrexone and NSP3 has the lowest binding energy (-11.8 kcal/mol) among the group of ligands docked with NSP3. This interaction showed the presence of three conventional hydrogen bonds – one hydrogen bond between hydroxybenzofuran group and amino acid residue THR 71, the second and third hydrogen bond between the furan group and amino acid residues ALA 38 and ASN 40; Six van der Waal's interaction between the benzene derivative and the different amino acid residues at VAL 49, GLY 48, PHE 132, ALA 39, LYS 44, HIS 45. Other interactions include pi-sigma interaction between the cyclopropylmethyl group and amino acid residue at ILE 131; amide-pi interaction between the isoquinoline derivative and amino acid residues at GLY 47, GLY 46 and pi- alkyl interaction between the methanobenzofuranyl group and amino acid residue ALA 50 (Fig. 4c).

Protein-ligand interactions between NSP9 RNA binding protein and Methylnaltrexone

Methylnaltrexone showed lowest binding energy (-11.3 kcal/mol) among the ligands that were docked with the NSP9 target. The interaction of the ligand with the protein residues showed that the ligand bonded with one conventional hydrogen bond between the hydroxybenzofuran group and amino acid residue MET 13; six van der Waals interaction was observed between the benzene derivative and different amino acid residues at PRO 58, SER 60, LYS 59, ILE 66, VAL 42 and THR 68 at the binding site (Fig. 4d).

Protein-ligand interactions between RNA dependent RNA polymerase and Ketazolam

Ketazolam is the ligand with lowest binding energy (-11.7 kcal/mol) among the ligands that were docked with RdRp target. The interaction of the ligand with the protein residues showed that the ligand bonded with two conventional hydrogen bonds between the oxazino group and amino acid residues GLY 432 and LYS 430. The ligand was involved in six van der Waals interactions between the phenyl groups and amino acid residues at SER 433, LYS 7, LEU 437, PHE 429, SER 4, and SER 1. (Fig. 4e).

Protein-ligand interactions between Replicase polyprotein 1a and Methylnaltrexone

Methylnaltrexone also had lowest binding energy (10.7 kcal/mol) among the ligands that docked with RP1a. The interaction of the ligand with the protein residues showed that the ligand bonded with two conventional hydrogen bonds. The first hydrogen bond interaction occurred between furan group and amino acid residue ARG 111, while the second hydrogen bond occurred between the carboxylic group and amino acid residue ARG 96. van der Waals interaction was observed between the phenyl group and amino acid residue THR 89. Other interactions observed between the ligand-target complex are pi-pi, which occurred between the phenyl groups of ligand and amino acid residue at PHE 92 and pi-alkyl, which occurred between the methyl group and amino acid residue at ILE 107 shown in (Fig. 4f).

Discussion

Drug repurposing approach through high throughput virtual screening, molecular docking and other computational techniques are widely used to understand protein-ligand interactions in the process of drug discovery [25].

The drugs hydroxychloroquine, lopinavir and remdesivir were used as standards because they have been arguably reported as off-label treatment for COVID-19 [26]. Hydroxychloroquine is an antimalarial drug that affects the endosomal function, blocks autophagosome-lysosome fusion and is being currently used for the management of COVID 19 in some countries of the world [27, 28]. Our study shows that hydroxychloroquine has inhibitory effect against all the SARS-CoV-2 targets studied and other studies have shown the effectiveness of hydroxychloroquine against the novel coronavirus. According to Wang et al., 2020, hydroxychloroquine was reported to be a blocker of ACE2 in SARS-CoV-2 [29]. Also, our study revealed that the most binding effect of hydroxychloroquine was on ACE2. However, studies recently carried out on the review of systematic clinical trials utilizing chloroquine (CQ) and hydrochloroquine (HCQ) as treatments of COVID-19, revealed that CQ and HCQ had very low potency which is comparable to placebo [44, 45].

Lopinavir is a wide spectrum antiviral drug that targets the viral protease and it is being considered in different combinations with ritonavir in a Phase IV clinical trial for pneumonia associated with COVID-19 [30]. In this study, it was observed that lopinavir had the best binding effect among the reference drugs tested, most especially against 3CLpro and ACE2. Similar observation has been reported by Uzunova, 2020 where lopinavir was shown as protease inhibitor, inhibiting 3Clike protease of SARS CoV-2 [26]. In addition, remdesivir is a viral RNA-dependent RNA polymerase inhibitor which has been shown to have activity against the SARS CoV-2 virus and is undergoing a Phase III level clinical trial [31]. This study also showed that remdesivir favourably bound to RdRp with very low binding energy (-7.5 kcal/mol).

This study has revealed over 100 compounds which further research works need to be carried out on so as to establish the efficacy of anyone that can be repurposed against SARS CoV-2 because they showed binding energies that are lower than some of the drugs which are currently being used for the management of Covid-19. Highly rated among these ligands are ethynodiol diacetate, ketazolam, methylnaltrexone and naloxone. The 4 high ranking drugs were generally observed to have fused rings. The common structural feature might have made the ligands to form similar favourable interactions with specific

amino acid residues on the target site. Methylnaltrexone and naloxone are similar both in structure and pharmacological activity [52]. Also, Methylnaltrexone and ethynodiol diacetate are both used as contraceptives, while ethynodiol diacetate and ketazolam can both act as sedatives [53].

Ketazolam, which showed the lowest binding energy with 3CLpro, is a benzodiazepine derivative with anxiolytic, anticonvulsant, sedative and skeletal muscle relaxant activity. It binds nonspecifically to benzodiazepine receptors which mediate sleep, affects muscle relaxation, anticonvulsant activity, motor coordination, and memory [32]. It interacted with only one amino acid residue - SER 158 with a conventional Hydrogen bond in 3CLpro. Ketazolam also gave the lowest binding energy against RdRp, showing interactions with two conventional hydrogen bonds at GLY 432, LYS 430; six van der Waals forces at SER 433, LYS 7, LEU 437, PHE 429, SER 4 and SER 1. Even though the mechanism of these interactions are yet to be understood, ketazolam may have a potential as an inhibitor for the SARS-CoV-2 virus, as it also showed lower binding energy in ACE2, NSP3, NSP9 and RP1a proteins.

Ethynodiol diacetate gave the least binding energy against ACE2. Ethynodiol diacetate is an oral contraceptive which may be used in combination with oestrogen. It is a synthetic progesterone, thus an agonist of progesterone receptor [33]. It interacted with ACE2 on amino acid residue with one conventional hydrogen bond - ILE 291, van der Waals interactions - PRO 415 and pi-alkyl interaction - PHE 438. It also showed interactions in 3CLpro, NSP3, NSP9, RdRp and RP1a.

Methylnaltrexone, which gives the lowest binding energy against ADP ribose of NSP3, NSP9 and RP1a, acts as a μ-opioid antagonist. It elicits its effects on the gastrointestinal tract to decrease opioid-induced constipation without producing analgesic effects or withdrawal symptoms [34]. It interacted with NSP3 using three conventional hydrogen bonds - ALA 38, ASN 40, THR 71; six van der Waals forces - VAL 49, GLY 48, PHE 32, ALA 39, LYS 44, HIS 45 and other interaction as pi-sigma at ILE 131; pi-alkyl at ALA 50 and Amide-Pi stacked at GLY 47, GLY 46. It interacted with NSP9 using three conventional hydrogen bonds at ASN 40, ALA 38, THR 71; six van der Waals forces at GLY 48, VAL 49, PHE 132, ALA 39, LYS 44, HIS 45 residues and other interactions as pi-sigma and amide-pi stacked. Also, it interacted with RP1a using two conventional hydrogen bonds - ARG 96, ARG 111; van der Waals forces - THR 89 and other interactions as pi-pi at PHE 92 and pi-alkyl at ILE 107. There must be an underlying activity that attracts Methylnaltrexone towards the amino acid residues at the active sites of the NSP3, NSP9 and RP1a.

The structural properties of ligands determine their physicochemical and biochemical properties. Physicochemical and biochemical properties in turn determine the pharmacokinetics and toxicity of the compounds. In drug repurposing, the pharmacological knowledge on the drug is important in its outlook for use for another disease. This is because providing the ADMET properties of the drug gives information on the current state of the drug for its original disease. However, if there is need for lead optimization to address the new disease the drug is being used for, the pharmacokinetic and ADME properties would need to be enhanced. Thus, building on the information obtained on the drug's use for previous disease would accelerate clinical development [46]. Lumacaftor was predicted to have good ADMET properties against SARS-CoV-2 [51], this was further validated with the result from this study. Methylnaltrexone, though a quaternary ammonium drug was predicted to have BBB permeability from our study, hence the need for further *in vitro* and *in vivo* ADMET studies of these drugs using animal models of SARS-CoV-2 to establish these findings. The drugs with the good ADMET properties and least binding energies could be optimized for use against Covid-19.

It was further observed that out of the first 38 compounds collated from the best 10 compounds under each target protein, only etravirine is an antiretroviral agent. The rest of the drugs are used for varieties of conditions. Their activities include antihistamine (rupatadine and buclizine), antipsychotic (pimozide), antifungal (butenafine), anticancer (regorafenib, bicalutamide, idarubicin and olaparib), contraceptive (drospirenone), antihypertension (spironolactone, netarsudil) and hormone replacement therapy in female and male (quinestrol, estrone and methyltestosterone) [20]. Of special interest in this study is olaparib which was observed to have the lowest bindingenergy on RNA dependent RNA polymerase (RdRp) protein target when compared to other targets of SAS-CoV-2 worked on; olaparib was not found among the first 20 drugs under other target proteins apart from the RdRp target. This observation is not out of order since the drug is an inhibitor of poly (ADP-ribose) polymerase (PARP) enzymes [35]. The PARP enzymes are involved in normal cellular homeostasis, such as DNA transcription, cell cycle regulation, and DNA repair [36]. Similarly, our target protein, RNA dependent RNA polymerase (RdRp) which enables the viral genome replication, has similar mode of mechanism of action as PARP [9]. This observation therefore justified the results predicted in this study. Hence, there is need for validation of the drugs predicted to be better than the reference drugs, off-label drugs used against COVID 19 at present. There is, therefore, need for further studies on the best four drugs, ketazolam, methylnaltrexone, ethynodiol diacetate and Naloxone, predicted and this should be done as a matter of urgency through *in vitro, in vivo* and clinical researches.

Conclusion

This study has predicted four drugs ketazolam, methylnaltrexone, ethynodiol diacetate and naloxone which displayed better binding energy and pharmacokinetic properties than the off-Wlabel reference drugs (hydroxychloroquine, lopinavir and remdesivir) which are currently investigated for the treatment of COVID-19. It was observed that these drugs bound the six target proteins - 3CLpro, ACE2, NSP3, NSP9, RdRp and RP1a studied. The various interactions observed, showed that these four drugs, whether individually or in combinational therapies are potential drugs against the SARS-CoV-2 targets. Thus, further investigations are recommended through preclinical and clinical studies to explore the potentials of these drugs and ascertain their effectiveness.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

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